

SUPPLEMENTAL DATA

Figure S1

Table S1

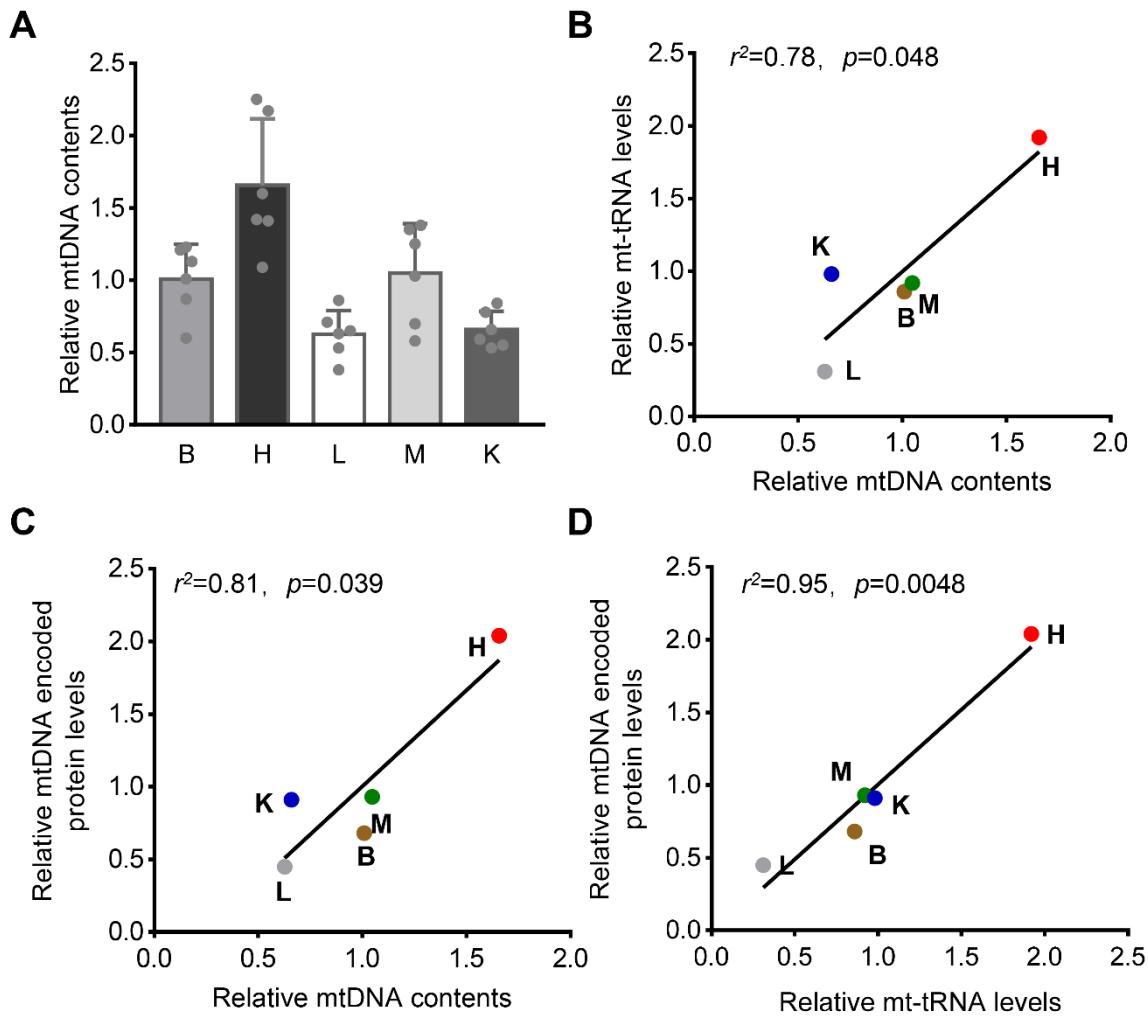
Table S2

Table S3

Table S4

Table S5

Table S6



Supplemental Figure S1. (A) Relative mtDNA contents of mice brain, heart, liver, skeletal muscle, and kidney. The relative levels were normalized to the mean values of mtDNA/nDNA ratio among five tissues. n = 6 per group. (B) The correlation between the average mtDNA contents and mt-tRNA levels of the five tissues. (C) The correlation between the average mtDNA contents and mtDNA encoded protein levels of the five tissues. (D) The correlation between the average mt-tRNA levels and mtDNA encoded protein levels of the five tissues. The r^2 and p values are indicated in each graph.

Supplemental Table S1. The steady state levels of mitochondrial tRNAs

| tRNAs | Mean value ± SD in different tissues | | | | |
|--------------------------|--------------------------------------|-----------|-----------|-----------------|-----------|
| | Brain | Heart | Liver | Skeletal Muscle | Kidney |
| tRNA ^{Phe} | 0.84±0.14 | 1.85±0.50 | 0.73±0.13 | 0.90±0.19 | 1.04±0.11 |
| tRNA ^{Val} | 1.05±0.06 | 1.83±0.15 | 0.31±0.04 | 0.76±0.09 | 1.06±0.10 |
| tRNA ^{Leu(UUR)} | 0.87±0.13 | 1.54±0.30 | 0.48±0.19 | 1.01±0.11 | 1.10±0.07 |
| tRNA ^{Ile} | 0.96±0.20 | 1.99±0.23 | 0.28±0.09 | 0.91±0.09 | 0.86±0.12 |
| tRNA ^{Met} | 0.72±0.17 | 2.20±0.35 | 0.25±0.03 | 0.98±0.14 | 0.85±0.30 |
| tRNA ^{Trp} | 0.90±0.16 | 1.69±0.26 | 0.34±0.18 | 1.02±0.03 | 1.05±0.13 |
| tRNA ^{Asp} | 0.86±0.17 | 1.90±0.17 | 0.27±0.07 | 0.98±0.20 | 0.99±0.11 |
| tRNA ^{Lys} | 0.84±0.11 | 2.25±0.28 | 0.31±0.06 | 0.83±0.10 | 0.77±0.27 |
| tRNA ^{Gly} | 0.83±0.14 | 1.99±0.05 | 0.21±0.10 | 0.98±0.19 | 0.98±0.11 |
| tRNA ^{Arg} | 0.79±0.16 | 2.12±0.41 | 0.37±0.08 | 0.82±0.15 | 0.90±0.20 |
| tRNA ^{His} | 0.87±0.19 | 2.34±0.29 | 0.20±0.04 | 0.75±0.12 | 0.84±0.13 |
| tRNA ^{Ser(AGY)} | 0.86±0.15 | 2.01±0.20 | 0.25±0.09 | 0.92±0.15 | 0.96±0.24 |
| tRNA ^{Leu(CUN)} | 0.78±0.06 | 2.00±0.10 | 0.30±0.03 | 0.94±0.06 | 0.97±0.09 |
| tRNA ^{Thr} | 0.88±0.37 | 2.14±0.40 | 0.26±0.08 | 0.80±0.17 | 0.92±0.21 |
| tRNA ^{Pro} | 1.05±0.20 | 1.81±0.08 | 0.41±0.10 | 0.79±0.08 | 0.94±0.18 |
| tRNA ^{Glu} | 0.85±0.03 | 1.71±0.37 | 0.58±0.25 | 1.04±0.12 | 0.81±0.02 |
| tRNA ^{Ser(UCN)} | 0.86±0.16 | 1.70±0.26 | 0.28±0.09 | 1.04±0.20 | 1.12±0.19 |
| tRNA ^{Tyr} | 0.93±0.12 | 2.03±0.34 | 0.21±0.06 | 0.87±0.16 | 0.96±0.45 |
| tRNA ^{Cys} | 0.61±0.24 | 2.31±0.30 | 0.29±0.05 | 0.79±0.18 | 1.00±0.17 |
| tRNA ^{Asn} | 0.92±0.18 | 1.84±0.18 | 0.29±0.05 | 0.96±0.24 | 0.99±0.05 |
| tRNA ^{Ala} | 0.79±0.13 | 1.72±0.16 | 0.37±0.13 | 1.03±0.11 | 1.09±0.19 |
| tRNA ^{Gln} | 0.96±0.19 | 1.73±0.17 | 0.37±0.07 | 0.98±0.09 | 0.97±0.10 |
| Average | 0.86±0.03 | 1.92±0.08 | 0.31±0.03 | 0.92±0.04 | 0.98±0.06 |

Relative levels of specific tRNA were normalized to the mean values of each tRNA among five tissues. The calculations were based on three to five independent experiments. The relative tRNA levels were indicated by mean value ± SD.

Supplemental Table S2. The aminoacylation levels of mitochondrial tRNAs

| tRNAs | Mean value ± SD in different tissues (%) | | | | |
|--------------------------|--|------------|-------------|-----------------|-------------|
| | Brain | Heart | Liver | Skeletal Muscle | Kidney |
| tRNA ^{Phe} | 74.60±0.91 | 68.66±2.12 | 75.32±0.19 | 90.14±2.01 | 72.64±1.73 |
| tRNA ^{Val} | 72.84±4.43 | 54.76±6.61 | 74.84±7.09 | 85.25±2.92 | 42.46±5.98 |
| tRNA ^{Leu(UUR)} | 35.55±1.78 | 59.36±3.26 | 63.65±13.42 | 72.68±9.67 | 21.27±3.84 |
| tRNA ^{Ile} | 59.47±3.95 | 71.36±0.54 | 75.85±5.00 | 79.33±10.63 | 47.14±10.63 |
| tRNA ^{Met} | 45.94±0.93 | 54.70±5.20 | 53.44±3.34 | 64.90±4.26 | 38.62±4.47 |
| tRNA ^{Trp} | 53.81±2.51 | 66.89±1.49 | 72.07±3.53 | 76.45±1.17 | 41.48±1.25 |
| tRNA ^{Lys} | 44.25±0.29 | 89.26±0.14 | 77.60±1.03 | 92.51±0.09 | 54.34±2.78 |
| tRNA ^{Gly} | 30.34±0.39 | 33.36±0.34 | 70.78±4.27 | 78.20±6.41 | 42.41±1.65 |
| tRNA ^{Arg} | 53.02±0.10 | 72.02±1.77 | 76.01±1.34 | 74.54±1.72 | 40.96±2.51 |
| tRNA ^{His} | 54.44±6.22 | 57.15±3.55 | 59.35±3.98 | 61.53±4.79 | 47.11±12.73 |
| tRNA ^{Leu(CUN)} | 52.57±2.16 | 71.15±2.94 | 84.06±5.29 | 84.77±4.61 | 36.59±9.38 |
| tRNA ^{Thr} | 60.30±0.42 | 62.76±4.50 | 65.89±3.32 | 67.08±2.48 | 50.80±4.21 |
| tRNA ^{Pro} | 15.19±2.86 | 11.74±6.77 | 55.84±0.78 | 68.89±2.08 | 11.16±0.48 |
| tRNA ^{Ser(UCN)} | 42.16±4.00 | 69.00±9.82 | 77.49±2.92 | 82.64±1.86 | 35.67±10.02 |
| tRNA ^{Tyr} | 41.22±1.96 | 46.22±1.47 | 38.08±1.18 | 72.72±2.69 | 27.63±0.00 |
| Average | 51.00±2.15 | 59.31±3.44 | 69.09±3.50 | 77.90±3.16 | 40.27±4.27 |

The calculations were based on two to three independent determinations. The aminoacylation levels of mitochondrial tRNAs were indicated by mean value ± SD.

Supplemental Table S3. The relative expression levels of *mt-aaRS* genes

| <i>Mt-aaRSs</i> | Mean value ± SD in different tissues | | | | |
|-----------------|--------------------------------------|-----------|-----------|-----------------|-----------|
| | Brain | Heart | Liver | Skeletal Muscle | Kidney |
| <i>Fars2</i> | 0.62±0.06 | 1.56±0.12 | 0.59±0.06 | 0.53±0.09 | 1.70±0.13 |
| <i>Vars2</i> | 1.05±0.09 | 1.17±0.17 | 0.63±0.07 | 0.51±0.12 | 1.65±0.15 |
| <i>Lars2</i> | 0.84±0.05 | 1.31±0.12 | 0.38±0.05 | 0.79±0.12 | 1.67±0.15 |
| <i>Iars2</i> | 0.86±0.05 | 1.29±0.06 | 0.53±0.09 | 0.59±0.12 | 1.72±0.13 |
| <i>Mars2</i> | 2.05±0.14 | 1.17±0.15 | 0.24±0.08 | 0.49±0.05 | 1.05±0.07 |
| <i>Wars2</i> | 1.01±0.07 | 0.76±0.08 | 0.45±0.05 | 0.61±0.08 | 2.17±0.08 |
| <i>Kars</i> | 1.03±0.04 | 1.41±0.10 | 0.59±0.12 | 0.79±0.09 | 1.19±0.06 |
| <i>Gars</i> | 1.89±0.14 | 0.89±0.06 | 0.50±0.07 | 0.59±0.08 | 1.14±0.07 |
| <i>Rars2</i> | 1.01±0.09 | 0.74±0.06 | 0.48±0.05 | 0.45±0.06 | 2.31±0.11 |
| <i>Hars2</i> | 0.77±0.04 | 1.34±0.18 | 0.38±0.04 | 0.75±0.14 | 1.75±0.20 |
| <i>Sars2</i> | 1.00±0.11 | 1.15±0.11 | 0.62±0.14 | 1.10±0.20 | 1.13±0.12 |
| <i>Tars2</i> | 1.10±0.10 | 1.33±0.16 | 0.61±0.10 | 0.68±0.10 | 1.29±0.09 |
| <i>Pars2</i> | 1.55±0.11 | 1.11±0.20 | 0.41±0.08 | 0.52±0.04 | 1.41±0.24 |
| <i>Yars2</i> | 1.13±0.10 | 1.24±0.07 | 0.68±0.11 | 0.57±0.07 | 1.37±0.06 |
| Average | 1.14±0.03 | 1.18±0.09 | 0.51±0.06 | 0.64±0.09 | 1.54±0.08 |

The relative levels were normalized to the mean values of each *mt-aaRS* gene among five tissues. n = 5 per group. The relative expression levels of *mt-aaRS* genes were indicated by mean value ± SD.

Supplemental Table S4. The relative expression levels of OXPHOS subunits

| Proteins | Mean value ± SD in different tissues | | | | | |
|------------------------|--------------------------------------|-----------|-----------|-----------------|-----------|-----------|
| | Brain | Heart | Liver | Skeletal Muscle | Kidney | |
| mtDNA encoded proteins | Nd1 | 0.89±0.04 | 1.20±0.13 | 0.70±0.08 | 1.08±0.07 | 1.12±0.06 |
| | Nd3 | 0.53±0.08 | 1.89±0.35 | 0.43±0.20 | 1.04±0.24 | 1.10±0.28 |
| | Co1 | 0.48±0.19 | 3.21±0.33 | 0.19±0.08 | 0.57±0.15 | 0.56±0.29 |
| | Co2 | 0.41±0.04 | 2.39±0.07 | 0.14±0.02 | 1.00±0.05 | 1.06±0.06 |
| | Atp6 | 1.15±0.06 | 1.35±0.03 | 0.65±0.13 | 0.99±0.13 | 0.86±0.03 |
| | Atp8 | 0.61±0.19 | 2.17±0.08 | 0.60±0.23 | 0.87±0.12 | 0.74±0.47 |
| nDNA encoded proteins | Ndufa9 | 0.97±0.03 | 1.57±0.19 | 0.57±0.06 | 1.00±0.12 | 0.89±0.17 |
| | Ndufb8 | 0.91±0.15 | 1.65±0.24 | 0.38±0.03 | 1.13±0.11 | 0.93±0.20 |
| | Sdha | 0.74±0.16 | 1.58±0.15 | 0.64±0.07 | 1.11±0.04 | 0.93±0.04 |
| | Sdhb | 0.73±0.08 | 1.90±0.18 | 0.73±0.18 | 0.78±0.15 | 0.86±0.16 |
| | Uqcrc2 | 0.88±0.18 | 1.94±0.22 | 0.64±0.08 | 0.76±0.23 | 0.78±0.21 |
| | Cox4 | 0.44±0.17 | 2.80±0.47 | 0.09±0.06 | 0.81±0.08 | 0.86±0.19 |
| | Atp5a | 0.74±0.07 | 2.24±0.37 | 0.54±0.08 | 0.77±0.15 | 0.71±0.21 |
| | Average | 0.73±0.01 | 1.99±0.11 | 0.49±0.05 | 0.92±0.04 | 0.88±0.05 |

Relative levels of specific protein were normalized to the mean values of each tRNA among five tissues. The calculations were based on three independent determinations. The relative expression levels of OXPHOS subunits were indicated by mean value ± SD.

Supplemental Table S5. Sequences of oligodeoxynucleosides for Northern blot analysis

| Probes | 5' DIG-oligodeoxynucleoside probe |
|-----------------------------|-----------------------------------|
| tRNA ^{Ala} | TAAGGACTGTAAGACTTCATCCTACATCTA |
| tRNA ^{Arg} | TTGGTAATTATGAACATCATCATAATCTAA |
| tRNA ^{Asn} | CTAGATTGGCAGGAATTAAACCTACGAAAAA |
| tRNA ^{Asp} | TAAGATATAGATTATTGATCTATAATT |
| tRNA ^{Cys} | AAGTCTTAGTAGAGATTCTCTACACCTTC |
| tRNA ^{Gln} | CTAGGACAATAGGAATTGAACCTACACTTA |
| tRNA ^{Glu} | TATTCTACACAGCATTCAACTGCGACCAA |
| tRNA ^{Gly} | TACTCTCTCTGGGTTATTCAGAATCTAC |
| tRNA ^{His} | GGTGAATAAGGAGGTTATTCCTGTTGTC |
| tRNA ^{Ile} | TAGAAATAAGAGGGCTTGAACCTCTATAAT |
| tRNA ^{Leu(UUR)} | TATTAGGGAGAGGATTGAAACCTCTGGGAA |
| tRNA ^{Leu(CUN)} | TACTTTATTGGATTGACCAAGGTTTT |
| tRNA ^{Lys} | TCACTATGGAGATTAAAGGTCTCTAACTT |
| tRNA ^{Met} | TAGTACGGGAAGGATTAAACCAACGTTTT |
| tRNA ^{Phe} | TGTTTATGGATACAATTATCCATCTAACGC |
| tRNA ^{Pro} | TCAAGAAGAAGGAGCTACTCCCCACCACCA |
| tRNA ^{Ser(UCN)} | TAAGAAAGCCATTTAAACATGGAAGC |
| tRNA ^{Ser(AGY)} | TAAGAAAGGAAGGAATCGAACCCCTAAAAA |
| tRNA ^{Thr} | TGTCTTGAGAAGAGAAGATCTTCATTCAG |
| tRNA ^{Trp} | CAGAAGTTAAACTGTGTGTTCTTAGGG |
| tRNA ^{Tyr} | TGGTAAAAGAGGATTAAACCTCTGTGTT |
| tRNA ^{Val} | TCAGAGTGGTCATTGGTCATGAAATCTCT |
| ct-tRNA ^{Asp(GTC)} | CTCCCCGTCGGGAATCGAACCCGGTCTC |
| ct-tRNA ^{Lys(CTT)} | CGCCTGAACAGGGACTTGAACCTGGACCC |
| ct-tRNA ^{Lys(TTT)} | CGCCCAACGTGGGCTCGAACCCACGACCC |
| 5S rRNA | GGGTGGTATGCCGTAGAC |

Oligodeoxynucleosides used for DIG-labeled probes were mice mitochondrial tRNAs, cytosolic tRNAs (ct-tRNA) and 5S rRNA.

Supplemental Table S6. List of primers for DNA and mRNA analysis

Primers for mtDNA/nDNA contents analysis

| Names | Forward Primer | Reverse Primer |
|-------|----------------------|----------------------|
| Mito | CTAGAAACCCCGAAACCAAA | CCAGCTATCACCAAGCTCGT |
| B2M | ATGGGAAGCCGAACATACTG | CAGTCTCAGTGGGGGTGAAT |

Primers for gene expression analysis

| Genes | Forward Primer | Reverse Primer |
|--------------|-------------------------|-------------------------|
| <i>Tars2</i> | TGTGCGACTGGCTGATTTC | CAAGGCTTGCTGTAGAACCTG |
| <i>Fars2</i> | GAGGAAGGTCAGTCATGCCTG | TCTGGGTGAGGTTAGTGTGGT |
| <i>Mars2</i> | ACCACACCTATCTTCTACGTGA | GCTGCAAAAACGGCAGAC |
| <i>Wars2</i> | CACATACCCTGTACTCCAGGC | GGACTTGGCAATGGAAAGAA |
| <i>Vars2</i> | ACATCCCTCTGTGGAGACTGG | GCAGCTAGGTCCCCATTTC |
| <i>Yars2</i> | GGGCGGATCAGATCAGTTGG | ATTGCCAGCAGACTTTCCCAG |
| <i>Gars</i> | GGAGGCAGCACTTATCCAAG | TCGGAAGCACTCTCCGTTCT |
| <i>Kars</i> | AGAACACAGCTAACCAAGACTGC | CGGCTTGACTTCGGATCTTGTA |
| <i>Pars2</i> | AGCTCCCTTGTGTTGTACC | GCCTACAGTAAGCATCACACAC |
| <i>Rars2</i> | AGAAGAGGTGGCAGATTTCAG | GCTGTCACCACTGTATCACACTT |
| <i>Iars2</i> | CGCCAGGGCAGGACATTTC | TTCCCTCCAGGTACAAGTCTG |
| <i>Lars2</i> | CATAGAGAGGAATTGCACCCCTG | GCCAGTCCTGCTTCATAGAGTTT |
| <i>Hars2</i> | CATAGAGAGGAATTGCACCCCTG | GCCAGTCCTGCTTCATAGAGTTT |
| <i>Sars2</i> | TGTGGTGGGTGAAAAGCCG | CCGTGCTGTAAGAGGGCTC |
| 18S rRNA | AGAAACGGCTACCACATCCA | CCCTCCAATGGATCCTCGTT |